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Endothelial cells as part of a vascular oxygen-sensing system: Hypoxia-induced release of autacoids

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Summary. Higher developed organisms are equipped with many central and local control mechanisms, which enable an adequate blood and oxygen supply to tissues over a wide range of demands. Global adaptive responses include changes in the circulatory and ventilatory system as well as increases in the oxygen carrying capacity of the blood. At the level of the specialized organs there exist additional control systems for the regulation of local blood flow. Most systems make use of highly specialized cells which are able to sense the oxygen partial pressure of the transport medium, blood, and within the tissues. In the past years, it has been shown that the vascular endothelium lining the entire circulatory system can actively modulate the vascular tone and platelet functions by the release of autacoids, among them prostacyclin and endothelium-derived nitric oxide (EDRF). Recent experiments demonstrate that the release of EDRF is P_{O_2}-dependent, which suggests that endothelial cells may act as functional local oxygen sensors within the vascular system.
Key words. Prostacyclin; EDRF; P_{O_2}; calcium; vascular oxygen sensitivity.

Introduction

It is of vital importance for the integrity and the function of cells that they receive a continuous supply of oxygen. It is therefore not surprising that the circulatory system makes use of various central and local control mechanisms which enable an adequate tissue oxygen supply to be maintained over a wide range of oxygen demands. In general, an increased oxygen consumption of organs, e.g. during exercise, as well as a reduced oxygen content of the transport medium, blood, leads by local vasodilation to an increase in regional blood flow. This vasomotor response is often combined with an increase in cardiac output and an augmented vascular resistance in other organs, which results in a redistribution of blood flow towards the hypoxic organ. This coordinated response to hypoxia reflects the function of effective mechanisms controlling tissue oxygen supply. An integral part of these control systems must be cells which directly or indirectly sense changes of oxygen tension of the blood or within the tissue and respond by the generation of signals which induce central or local responses of the circulatory system.

Oxygen-sensing structures and their localization

Highly developed organisms possess very specialized cells which are able to sense the oxygen partial pressure of the transport medium, blood. These cells are involved in the adaptive responses of the circulatory system and the ventilatory system, and in the increase in the oxygen-carrying capacity of the blood. Chemoreceptors in the carotid bodies, located in the bifurcation of the carotid arteries, and in the aortic arch, respond to changes in oxygen tension by activating afferent nerve fibers [14]. This increased activity stimulates ventilation and contributes to a concentration of the blood volume in vital organs. Likewise, the skeletal muscle contains elements which induce an enhanced afferent neural activity during regional hypoxemia, resulting in an augmented cardiac output and a peripheral vasoconstriction in non-hypoxic organs [25]. It should also be briefly mentioned that under conditions of hypoxemia, specialized, but not yet identified kidney cells release the glycoprotein erythropoietin which stimulates an enhanced production of erythrocytes in the bone marrow [3].

In parallel to these global control systems there exist local mechanisms which regulate the matching of oxygen demands and oxygen supply within an individual organ. The nature of the oxygen-sensitive structures acting at the local tissue level is not completely understood. There is convincing evidence that parenchymal cells, under conditions of hypoxia, release increased amounts of metabolites such as adenosine, lactate, hydrogen ions and potassium ions, which accumulate in the tissue. These metabolites induce relaxation of the vascular smooth muscle resulting in an increased blood flow in the hypoxic area [4]. This mechanism, however, implies that the cells must suffer from a certain degree of hypoxia before the organ blood flow will be affected. Another principle of control could be located in the arterial vessels itself, allowing a more rapid response to changes of blood oxygen tension without involvement of parenchymal cells. This presupposes that blood vessels are directly affected if the oxygen content or tension of the blood is reduced, either as a result of hypoxemia or owing to an augmented extraction of oxygen.

There is indeed ample evidence that blood vessels react with a dilation (i.e. increase in organ blood flow) to a reduction of oxygen tension even in the absence of tissue-derived metabolites. In arterioles of the cheek pouch of the hamster, Jackson and Duling [21] demonstrated a direct effect of oxygen on vascular diameters that was independent of flow conditions or the presence or absence of adjacent parenchymal tissue. Likewise, in canine skeletal muscle, vasodilation in response to a sudden onset of hypoxemia occurred as soon as the hypoxic blood reached the organ, while there were still no detectable changes in tissue oxygenation. Therefore it was concluded that the observed dilation resulted from a direct action of oxygen on the vasculature of the skeletal muscle [36]. These findings, in combination with numerous observations in isolated blood vessels [10, 11], demonstrate direct effects of oxygen on elements of the vascular wall which might contribute to the local control of vascular tone. Endothelial cells which are in direct contact with the flowing blood have a number of properties which predestinate them as effective functional vascular oxygen sensors.

Vasomotor function of the endothelium

The vascular endothelium lining the entire circulatory system is a highly specialized tissue. It is involved in the modulation of immune responses [19] and of vascular cell growth [12], and in the regulation of the level of hemostatic [43], inflammatory [5], and vasoactive [35] agents in the blood. In addition, the endothelium releases autacoids (tissue hormones) which decisively affect vascular tone and platelet function. Many endothelium-derived autacoids have been described and characterized, among them prostaglandins [30], platelet activating factor [44], the vasoconstrictor peptide endothelin [45] and the potent vasodilator compound EDRF (endothelium-derived relaxant factor [8, 16]), which is identical with nitric oxide [33]. EDRF stimulates the soluble guanylate cyclase in vascular smooth muscle directly [31] and induces vasodilation by cGMP-dependent mechanisms. Many compounds, e.g. platelet-derived substances like serotonin and ATP, and peptides like bradykinin or substance P [8], elicit a release of EDRF by specific receptor-dependent pathways. The release of EDRF results in an endothelium-dependent vasodilation which often counteracts simultaneous direct constrictor effects of these compounds on vascular smooth muscle [2]. Most of these stimuli also induce the release of prostacyclin (PGI_2) from endothelial cells. PGI_2 elicits an increase of cellular cAMP by a receptor-dependent stimulation of adenylate cyclase. It is a potent antiaggregatory substance, and also induces vasodilation at appropriately high concentrations [30]. However, not all vessels are sensitive to PGI_2 [15]. Since the endothelial autacoid release is not only stimulated by receptor-dependent stimuli but also by a number of physical stimuli, it was hypothesized that endothelial cells may also respond specifically to changes in oxygen tension.

Hypoxia induces endothelium-dependent vasodilation

The effects of hypoxia on the vasomotor function of endothelial cells were studied in isolated arteries of different species. The arterial segments were cannulated at both ends and suspended in an organ bath which was gassed to establish a P_{O_2} of more than 300 mm Hg. The arterial lumen was perfused separately from the organ bath either at a P_{O_2} of 130 mm Hg (normoxia) or of 20–40 mm Hg (hypoxia). This approach differs from others where endothelium and vascular smooth muscle are simultaneously made hypoxic, since only the endothelium is exposed to the reduced P_{O_2}, whereas at the adven-

titia a P_{O_2} of more than 300 mm Hg is maintained throughout the experiment.

It is consistent with a role of endothelial cells as vascular oxygen sensors that precontracted arterial segments with an intact endothelium responded to luminal hypoxia with a vasodilation, whereas this dilator response was abolished after endothelial denudation (fig. 1)[7,38]. This endothelium-dependent vasodilation in response to luminal hypoxia is associated with an enhanced release of PGI_2 and of EDRF[7,38]. In rat tail arteries, which exhibit a pronounced sensitivity to this prostanoid, PGI_2 could be identified as the main mediator of this hypoxic vasodilator response. In all other arteries studied so far, EDRF mediates the hypoxic vasodilation; EDRF-blocking substances like oxyhemoglobin, which scavenges EDRF[29] and blocks endothelium-dependent dilation in vitro but not in vivo, abolished the hypoxia-induced dilation in these segments. Moreover, in bioassay experiments, the release of an EDRF-like vasodilator compound from vascular segments as well as from cultured endothelial cells could be demonstrated during superfusion with low P_{O_2}[38].

In contrast, no release of either of the autacoids can be detected under true *anoxic* conditions. This may be explained by the fact that the synthesis of EDRF from l-arginine[34], as well as that of PGI_2 from arachidonic acid[24], involves the incorporation of molecular oxygen. During true anoxia, even vasoconstriction has been described[6,41]; this is apparently not only due to the lack of formation of endothelial dilator autacoids but also to the release of endothelium-derived constricting factor(s)[41] as well as potassium ions and norepinephrine[6]. Changes of oxygen tension not only affect the release, but also the biological half-life activity of EDRF[8]. Hyperoxia results in an enhanced generation of oxygen free radicals, predominantly of superoxide anions, which inactivate EDRF[42]. This effect may contribute to the reduced organ blood perfusion which is often observed under hyperoxic conditions. This vasoconstriction leads to a paradoxical impairment of tissue oxygen supply during hyperoxia[28].

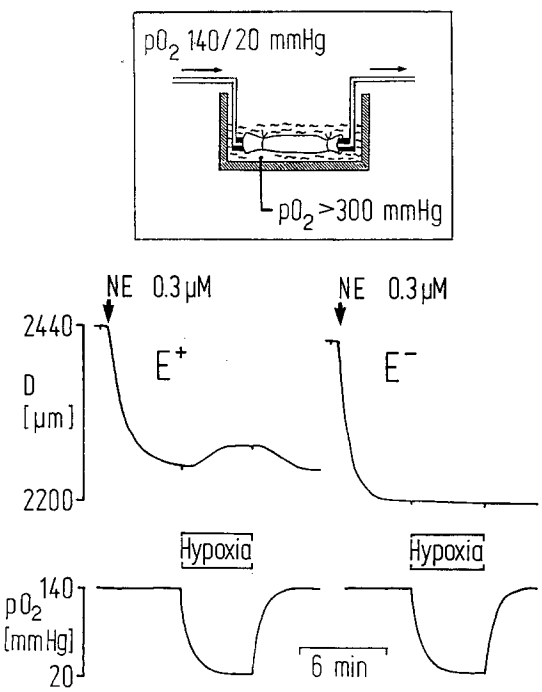

Figure 1. Endothelium-dependent dilator response of canine femoral artery segments to selective luminal hypoxia.
Top: Schematic diagram of the experimental setup which allows selective induction of hypoxia at the endothelial surface. Middle: Diameter recordings (D) of a canine femoral artery segment (precontracted with norepinephrine (NE)) with and without endothelium. Dilation is only observed in the presence of the endothelium. Bottom: Changes of luminal P_{O_2} as recorded in the inflow cannula.

Recent experiments suggest that the endothelium might generate additional vasodilator signals in response to hypoxia. In canine carotid arteries during stepwise reduction of P_{O_2}, a progressive, endothelium-dependent hyperpolarization of vascular smooth muscle cells was demonstrated[17]. Its functional significance, and its dependence on the release of a recently identified endothelium-derived hyperpolarizing factor[18], are not yet clear.

Potential mechanisms of endothelial oxygen-sensing

The experimental evidence is consistent with a role of endothelial cells as oxygen sensors in the vascular wall, since they respond in a P_{O_2}-dependent way with the release of vasoactive autacoids. The primary oxygen-sensing process within the endothelial cells has not yet been clarified. It has been inferred that in hypoxic cells, there is a reduction of intracellular ATP which might affect especially ATP-dependent processes at the cell membrane. In liver cells, experimental findings suggest the existence of intracellular ATP gradients and a relative lack of ATP at the cell membrane when the cellular production of ATP is reduced[1]. In endothelial cells, a reduced level of ATP could result in an increase of the PGI_2-precursor arachidonic acid since the reacylation of this compound to form membrane glycerophospholipids is dependent on ATP. In cultured bovine aortic endothelial cells, however, we did not observe either reduced oxygen consumption or a decrease of cellular ATP content after a 30-min exposure of the cells to superfusion at a P_{O_2} of 25 mm Hg (fig. 2).

An alternative hypothesis is based on the observation that in the effluent of hypoxically perfused organs, even increased amounts of ATP[20] could be detected. Since several adenine nucleotides are potent stimulators of the endothelial autacoid release[9,26], it is conceivable that the enhanced release of EDRF and PGI_2 is due to the action of a hypoxia-induced release of ATP on endothelial cells. In cultured aortic endothelial cells, however, the blockade of the endothelial ATP (P_{2y})-receptors by reactive blue did not affect the hypoxia-induced release of PGI_2 (fig. 3). It also remains unclear how hypoxia could induce specifically the release of ATP from intact cells, and what could be the main source of ATP in the hypoxic tissue. At present, perhaps the most important finding is the

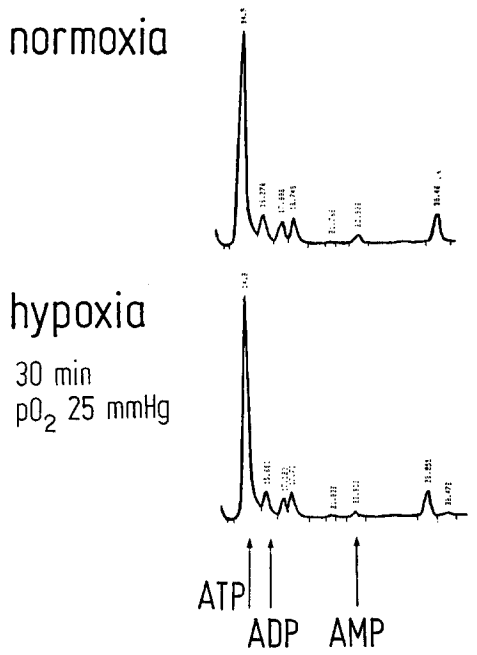

Figure 2. Distribution of cytosolic ATP, ADP, and AMP (HPLC analysis) in cultured bovine endothelial cells after 30 min superfusion with normoxic (P_{O_2} 140 mm Hg) or hypoxic (P_{O_2} 25 mm Hg) Tyrode's solution. 30 min of hypoxia did not induce any detectable changes.

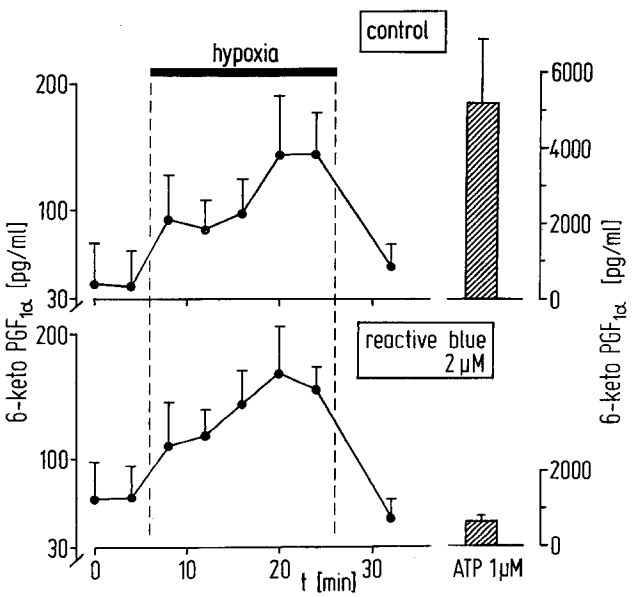

Figure 3. Lack of effect of the P_{2y}-receptor blocking compound reactive blue on the hypoxia (P_{O_2} 24 ± 6 mm Hg)-induced release of PGI_2 (measured as 6-keto $PGF_{1\alpha}$). Reactive blue virtually abolishes the ATP-induced (bars) but not the hypoxia-induced release of PGI_2, indicating that hypoxic response is not due to an effect of released ATP (n = 6) (X ± SEM).

observation that hypoxia induces an increase of intracellular free Ca^{2+} in cultured porcine and aortic endothelial cells[27]. Since the synthesis of PGI_2 as well as that of EDRF is Ca^{2+}-dependent, this increase in intracellular Ca^{2+} might represent an essential step in the signal cascade transducing the signal 'P_{O_2}' acting on endothelial cells into an enhanced release of endothelial autacoids. This holds especially true for EDRF, since the EDRF forming enzyme is directly dependent on Ca^{2+} [9a]. In fact, the removal of extracellular Ca^{2+} abolishes the EDRF-induced hypoxic vasodilation of isolated arteries[38].

Physiologic implications of hypoxia-induced endothelial autacoid release

Since cultured and native endothelial cells respond to a decrease in partial pressure of oxygen by an enhanced release of vasodilator autacoids, endothelial cells behave functionally as oxygen sensors within the vascular wall. Due to the experimental difficulties, this endothelial oxygen-sensor function has not yet been conclusively demonstrated under in vivo conditions. It is, however, interesting to note that prostaglandins appear not to be involved in the oxygen reactivity of microvessels[22], whereas lipoxygenase inhibitors, which are also unspecific inactivators of EDRF, abolished the oxygen reactivity of hamster arterioles in vivo[23]. Since stereospecific inhibitors of the EDRF synthesis[32] have now been shown to be effective in the microcirculation[39] a more detailed investigation of the role of EDRF in the blood flow responses to hypoxemia in vivo should be possible. The available data on in vitro experiments suggest that endothelium-dependent dilations may significantly contribute to the local P_{O_2}-dependent regulation of blood flow, and may represent a major biological equivalent of vascular oxygen reactivity. An endothelial oxygen sensor in the vascular wall enables the vasculature to respond rapidly to decreases in arterial P_{O_2} without the need for previous accumulation, with the potential risk of hypoxia-induced parenchymal cell damage. Furthermore, the hypoxic signal can be effective in conduit arteries which are not reached by tissue metabolites although they can contribute considerably to the overall vascular resistance of an organ. In addition, endothelial cells may not only respond to acute changes of oxygen tension but may be under a constant stimulatory influence of the reduced P_{O_2} existing in small arteries and arterioles[13]. This may have important implications for the balance of the vasomotor tone in these vessels and for the adjustment of tissue perfusion. Only recently, it has been shown that the inhibition of EDRF release by 1-nitro-arginine induced a significant reduction of arteriolar diameters in the hamster skin[39]. Consistent with this is the observation that similar inhibitors of EDRF synthesis induced a hypertensive response in rabbits[40]. Moreover, in rabbit hind-limb skeletal muscle, the oxygen partial pressure distribution deteriorates significantly after blockade of EDRF release[37] and obviously, the expected accumulation of tissue metabolites is unable to compensate for the impairment of nutritive tissue perfusion.

Conclusion

In conclusion, recent in vivo findings suggest a decisive role of endothelial cells in the modulation of vascular

tone in resistance vessels. Since EDRF synthesis and stability are P_{O_2}-dependent, the endothelium may well represent an effective vascular oxygen-sensing system which responds to acute and chronic changes of the P_{O_2} of the blood with potent vasoactive signals.

Acknowledgment. The author thanks Dr A. Ogilvie, Erlangen, for the HPLC-determinations of cytosolic nucleotide fractions. This work was supported by the DFG (Po 307/1-1).

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